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Microbiological Evaluation of Ground Beef Treated with Selected Medical Plants Volatile Oils

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Abstract: This investigation was carried out to study the possibility of using some natural substances as volatile oils substituted of synthetic chemical for improvement shelf- life of ground beef. Minced beef treated with some essential oils (cumin, black seeds, marjoram, rosemary and sage) with different levels0.25 and 0.50 %, and control sample (without any additions). The treated meat samples were stored at 4±1º C up to 15 day. Addition of volatile oils to minced meat reduced the total bacterial counts compared with control sample. Pyschrotrophic bacteria counts in all treatments at zero time were similar, but there is significant ($p \geq 0.05$) difference between samples with progressive of storage periods. Pyschrotrophic bacteria counts reduced with addition of essential oil compared with control sample. Staphylococcus aureus counts at zero time was higher in control sample (4.30 104 CFU/g) compared with other treated minced beef samples contained essential oils and which ranged between 2.7 to 3.69 104 CFU/g. With progressive of storage period, Staphylococcus aureus counts was increased to 6.15 104 CFU/g in control sample. Whereas, Staphylococcus aureus counts was reduced and disappear in minced beef samples contained volatile oils as a results of inhibitory effects of antimicrobial substances. Colliform group counts at zero time were higher in control sample (4.50 104 CFU/g) in compared with other minced beef samples which ranged between 2.45 to 3.59 104 CFU/g in minced beef samples contained essential oils. Colliform group counts was significant ($p \geq 0.05$) increased to 8.65 104 CFU/g in control sample with progressive of storage periods.. Whereas, their counts were reduced and disappear in minced beef samples contained volatile oils cause with inhibitory effects of these substances. All treatments of minced beef were free from Salmonella at zero time or throughout the storage periods.
Keywords: Minced beef, Essential oils, Antimicrobial activity.

1 Introduction

Minced beef is appreciated because of its convenience. Unfortunately, its shelf- life is limited because the large exposed surface area facilitates spoilage. The rate of deteriorative changes depends on meat composition, hygienic practices during cutting, grinding, and preparation and storage conditions. The most important factor in controlling meat spoilage is microbial contamination and their growth, which can effects on minced meat safety and its color [6]. Minimizing product contamination and delaying or inhibiting growth of spoilage and pathogenic organisms in the product are major keys for improving fresh meat shelf- life and increasing consumer safety [20].Essential oils (Eos) are volatile, natural and complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites. In addition to being used as flavoring agents in foods, Eos exhibit antibacterial, antifungal and antioxidant properties [5]. The International Organization for Standardization (ISO) defines Eos as products obtained from plant parts by steam drag distillation; in general, EOs, are complex mixtures of volatile lipophilic substances [18]. Plant extracts represent a rich potential source of alternative and environmentally acceptable control agents for infectious organisms due to their antimicrobial properties. Plants possess essential oils, which could be utilized for killing microorganisms [4]. In vitro studies have reported the high efficiency of biocidal Eos on pathogens that cause food poisoning and on spoilage bacteria [8,18]. The use of essential oils which present a better choice than some synthetic chemical additives, especially for ”organic” and ”natural” food production, which has become popular mostly in the Western society, and is widely accepted by consumers [21]. The antimicrobial properties of Eos from herbs have increased interest in the possibility of forming an alternative for responding to consumer demands for the use

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of natural additives in foods [12]. Thus, the main objective of the current study was to evaluate the influence of a treatment of minced meats with selected natural substances as volatile oils to improve the shelf-life on minced beef.

2 Materials and Methods

2.1 Meat

Fresh beef (bottom round) was purchased from local market at Assiut city, Egypt and transported under refrigeration to the laboratory within 30 min. Then, meat was cut and minced with a grinder through a 4 mm plate diameter (AC110V, China) just before analysis and treatments.

2.2 Extraction of the Essential Oil

Essential oils were obtained by steam distillation method as described by [11]. The plant material were Cumin (Cuminumcyminum), Black seeds (Nigella sativa), Marjoram (Origanummajorana), Rosemary (Rosmarinus officinalis) and Sage (Salvia officinalis). About 300g, was cut into small pieces, and placed in a flask (4L) together with doubly distilled water (1.5L). The mixture was boiled for 3h, collected essential oil were dried with anhydrous sodium sulfate and kept at –18 C until its use.

2.3 Preparation of Minced Beef Treated with Essential Oils

Minced beef was mixed in sterile mixer with essential oils (0.25 and 0.5%) of their weight, The samples were divided into two groups; the first group was treated with either 0.25 or 0.5% essential oils, second group was kept as a control group (without any additions). Each sample was packed in polyethylene bags and stored at 4°C ± 1 , and all analysis was conducted at intervals of 0, 3, 6, 9, 12 and 15 days.

2.4 Microbiological Methods

2.4.1 Sample Preparation

Ten grams of each sample were mixed with 90 ml of sterile saline solution (9 g NaCl/ 1L distilled water) in a blender, under sterile conditions to give 1/10 dilution. Serial dilutions were prepared to be used for counting several types of bacteria.

2.4.2 Total Bacterial Count

The total bacterial counts were determined using the plate counts technique on a nutrient agar medium according to procedures by [1, 7]. The plates were incubated at 37oC for 48 h.

2.4.3 Psychotropic Bacterial Count

Psychotropic bacterial count was carried out as described in typical procedure of the plat bacterial count methods, except, incubation was achieved at 8oC for 5 days according to [1]

2.4.4 Staphylococcus Aureus Count

The Staphylococcus aureus was determined using Manitol salt agar medium according to the method described by [7]. The plates were incubated at 35±2o C for 24 to 48 h.

2.4.5 Coliform Group Bacteria Count

Coliform group bacteria were determined using Mac Conkey agar medium according to the procedures described by [1, 7]. The plates were incubated at 37oC for 24 h.

2.4.6 Detection of Salmonella

The presence or absence of Salmonella was determined according to the method described by [9], Salmonella Shigella agar plates were incubated at 35o C for 24 h. Salmonella appeared as black colonies, some of them with metallic sheet.

2.5 Statistical Analysis

Data were analysed by analysis of variance (ANOVA) using a completely randomised factorial design. Basic statistics and ANOVA were performed to test the significance within replications and between treatments [14]. (L.S.D) tests were used to determine the differences among means at the level of 0.05%.

3 Results and Discussion

3.1 Changes in total Bacterial Count of Minced Beef Treated with Essential Oils during Storage at 4 ±1°C for 15 Days

Data presented in Table (1) pointed to the changes in total bacterial counts in minced beef samples formulated with essential oil of cumin, black seeds, marjoram, rosemary and sage under levels 0.25 and 0.50 % during storage at 4±1°C up to 15 days was ranged between 6.34 and 6.46 log cfu/g at zero time. Meanwhile, the total bacterial count in control sample was 6.70 log cfu/g. At the end of storage periods the final total bacterial counts in samples contained essential oil of cumin were (6.42 and 5.89), black seeds (8.34 and 7.31), marjoram (5.34 and 4.46), rosemary (6.46 and 6.02) and sage (4.09 and 3.95) log cfu/g under levels 0.25 and 0.50 %, respectively.

On the other side, total bacterial counts for treated samples were lower than the control sample (10.53 log cfu/g). The relatively high initial counts of control samples may be attributed to the grinding process, which compounds the problem by introducing the pathogens into the interior of the meat and contributes to the increase of total viable counts of meat [15].From these results, it could be observed that the addition of marjoram and sage essential oils under level 0.25 and 0.50 % to the minced meat caused highest significant ($p \geq 0.05$) decrease in total bacterial count, this decrease might be attributed to the effect of the antimicrobial compounds in these essential oils. Such findings are in agreement with those reported by [19] they found that the essential oil of sage has antibacterial activity due to the presence of 1.8-cineol compound.

Table 1: Changes in total bacterial count (104cfu/g) of minced beef treated with essential oils during storage at 4±1°C up to 15 days:

Treatments		**Storage periods (days)**						
		0	**3**	**6**	**9**	**12**	**15**	**Mean**
Control		6.70	6.48	6.26	7.37	8.21	10.53	7.59
Cumin oil 0.25 %		6.46	6.45	5.36	5.89	6.36	6.42	6.15
Cumin oil 0.50 %		6.42	6.40	5.28	4.32	5.30	5.89	4.39
Black seeds oil 0.25 %		6.42	6.35	5.44	6.40	7.43	8.34	6.73
Black seeds oil 0.50 %		6.38	6.21	5.38	6.36	6.40	7.31	6.34
Marjoram oil 0.25%		6.45	6.21	5.27	6.15	5.38	5.34	5.80
Marjoram oil 0.50 %		6.45	5.48	5.30	5.10	4.01	4.46	5.13
Rosemary oil 0.25 %		6.45	6.36	5.30	6.38	6.45	6.46	6.23
Rosemary oil 0.50 %		6.41	6.31	5.28	6.32	6.41	6.02	6.12
Sage oil 0.25 %		6.40	4.27	4.18	4.15	4.04	4.00	4.50
Sage oil 0.50 %		6.34	4.26	4.11	4.12	3.61	3.95	5.60
Mean		6.44	5.89	5.19	5.6	6.24	5.78	5.87
L.S.D $_{0.05}$	**A**	**0.05**						
	B	**0.07**						
	AB	**0.17**						

A= Treatments (in the same column), **B**= Storage period (in the same row), **AB**= Interaction

3.2 Changes in Psychrotrophic Bacterial Count of Minced Beef Treated with Essential Oils during Storage at 4±1°C up to 15 days

The obtained data in Table (2) revealed that, the psychrotrophic bacterial count in control sample was 6.30 104cfu/g at zero time and significant ($p \geq 0.05$) difference increased to 11.46 104cfu/g at the end of storage periods. Meanwhile, the psychotropic bacterial count of samples contained essential oil of cumin, black seeds; marjoram, rosemary and sage under levels 0.25 and 0.50 % had ranged from 5.10 to 6.14 104cfu/g at zero time, while at the end of storage periods showed little changes in psychrotrophic bacterial count under the same condition.

From the same data it could be stated that minced beef samples treated with essential oils had little number of psychrotrophic bacterial count compared with control sample. On the other hand, the increasing rate of psychrotrophic bacterial count was slow in minced beef samples treated with essential oils as well as cumin and black seeds than control sample during storage periods at4 ±1°C up to 15 days. However, in case of marjoram, rosemary and sage oils the total psychrotrophic bacterial count was decreased as the storage periods increased. These results agree with those reported by [3], they found that addition of rosemary and sage extracts under level 0.1 % to minced beef had inhibitory effect against psychrotrophic bacteria as compared to the control sample. [9], reported

that plant extracts and essential oils constitute a natural source of antimicrobial mixtures or pure compounds for centuries. Essential oils and purified components are used as natural prevent the growth of food borne bacteria and molds antimicrobials in food systems, as well as to resulting in extension of the shelf- life of processed foods.

3.3 Changes in Staphylococcus Aureus Counts of Minced Beef Treated with Essential Oils during Storage at 4-±1°C up to 15 days.

Data given in Table (3) shown the Staphylococcus aureus counts in different minced beef contained essential oils of cumin, black seeds, marjoram, rosemary and sage under levels 0.25 and 0.50 % and control sample during storage at 4±1°C up to 15 days. The results showed that Staphylococcus aureus counts increased in control sample so, its counts at zero time was 4.3 104cfu/g reached to 6.15 104cfu/g at the end of storage periods. Moreover, from the same data, it could be noticed that Staphylococcus aureus counts significant ($p \geq 0.05$) decreased during storage time progress of minced beef samples contained essential oils under level 0.25 and 0.50 %. Staphylococcus aureus counts for these samples were less than the control sample. Besides, after storage for 6 days St. aureus disappeared in minced beef samples contained cumin and marjoram essential oils under level 0.50%.

Also, Staphylococcus aureus disappeared in samples contained cumin and marjoram essential oils (0.25 and 0.50 %) , rosemary and sage essential oils under level 0.50 % at 9 days storage. After 12 days of the refrigerated storage, there is no any St. aureus cells in all studied samples except that contained black seeds oil at 0.25 % and the control. The disappearing of St. aureus may be attributed to antimicrobial activity of these essential oils [2].

Table 2: Changes in psychrotrophic bacterial count (104cfu/g) of minced beef treated with essential oils during storage at 4±1°C up to 15 days.

Treatments		**Storage periods (days)**						
		0	**3**	**6**	**9**	**12**	**15**	**Mean**
Control		6.30	6.44	7.39	9.56	10.77	11.46	8.65
Cumin oil 0.25 %		6.14	6.28	6.69	7.15	7.40	8.10	6.96
Cumin oil 0.50 %		6.08	6.00	6.30	6.49	6.90	7.15	6.48
Black seeds oil 0.25 %		5.84	6.32	5.29	6.24	6.36	6.36	4.39
Black seeds oil 0.50 %		5.10	4.46	4.11	4.00	3.83	3.49	4.16
Marjoram oil 0.25%		5.61	4.96	4.31	3.91	3.63	3.45	4.31
Marjoram oil 0.50 %		6.20	6.32	6.00	5.24	5.10	4.69	5.59
Rosemary oil 0.25 %		6.11	5.63	5.81	5.15	4.91	4.85	5.41
Rosemary oil 0.50 %		5.26	5.10	4.83	4.55	4.36	4.11	4.70
Sage oil 0.25 %		5.91	5.43	4.93	4.66	4.32	4.18	4.90
Sage oil 0.50 %		5.13	4.96	4.65	4.13	3.86	3.66	6.06
Mean		5.78	5.62	5.59	5.55	5.58	5.48	5.60
L.S.D $_{0.05}$	**A**	**0.07**						
	B	**0.09**						
	AB	**0.22**						

A= Treatments (in the same column), **B** = Storage period (in the same row), **AB**= Interaction

3.4 Changes in Coliform Group Counts of Minced Beef Treated with Essential Oils during Storage at 4±1°C up to 15 days.

From the obtained data, it observed that control sample had significantly ($p \geq 0.05$) difference the higher counts coliform group compared to other treatments, which formulated with essential oils under levels 0.25 and 0.50 % at zero time and at end of storage periods (Table 4). Essential oil of sage under level 0.50 %was more active of inhibiting effect against coliform group bacteria, which disappear after 6 days of storage periods compared with other essential oils. Moreover, it could be noticed that the coliform group disappear in most minced beef samples formulated with essential oil under levels 0.25 and 0.50 % after 9 days. These results indicated that the essential oils at both concentrations inhibited the growth of coliform group bacteria. These results are similar as mentioned by [10], they tested the essential oils of sage, rosemary, caraway cumin, clove and thyme and their constituents, for their inhibitory effect against three Gram-negative bacteria (Pseudomonasfluorescens, Escherichia coli, and Serratia marcescens) and four Gram-positive bacteria (Staphylococcus aureus, Micrococcus spp., Saracina spp. and Bacillus subtilis), very low concentration (0.25- 12 mg/ml)of the various oils were sufficient to prevent microbial growth.

Table 3: Changes in Staphylococcus aureus counts (104cfu/g) of minced beef treated with essential oils during storage at 4±1°C up to 15 days.

Treatments	**Storage periods (days)**						
	0	**3**	**6**	**9**	**12**	**15**	**Mean**
Control	4.30	4.90	5.17	5.55	5.81	6.15	5.31
Cumin oil 0.25 %	3.13	2.85	2.50	0.00	0.00	0.00	1.41
Cumin oil 0.50 %	2.70	2.40	0.00	0.00	0.00	0.00	0.85
Black seeds oil 0.25 %	3.91	3.60	3.41	3.11	2.69	0.00	2.79
Black seeds oil 0.50 %	3.43	2.53	2.25	2.00	0.00	0.00	1.70
Marjoram oil 0.25%	3.23	3.01	2.59	0.00	0.00	0.00	1.47
Marjoram oil 0.50 %	2.96	2.43	0.00	0.00	0.00	0.00	0.90
Rosemary oil 0.25 %	3.69	2.51	2.11	2.05	0.00	0.00	1.73
Rosemary oil 0.50 %	3.47	2.95	2.00	0.00	0.00	0.00	1.40
Sage oil 0.25 %	3.54	3.39	2.53	2.13	0.00	0.00	1.93
Sage oil 0.50 %	3.06	2.43	2.15	0.00	0.00	0.00	1.27
Mean	3.40	3.00	2.25	1.35	0.77	0.56	1.88
L.S.D $_{0.05}$ **A**	0.07						
B	0.10						
AB	0.24						

A= Treatments (in the same column), **B** = Storage period (in the same row), **AB**= Interaction

Table 4: Changes in coliform group counts (104cfu/g) of minced beef treated with essential oils during storage at 4±1°C up to 15 days.

Treatments	Storage periods (days)						
	0	**3**	**6**	**9**	**12**	**15**	**Mean**
Control	4.50	4.90	5.25	6.55	7.40	8.65	6.21
Cumin oil 0.25 %	3.27	3.05	2.85	2.46	0.00	0.00	1.94
Cumin oil 0.50 %	3.11	2.73	0.00	0.00	0.00	0.00	0.97
Black seeds oil 0.25 %	3.59	3.29	2.50	0.00	0.00	0.00	1.56
Black seeds oil 0.50 %	3.23	2.90	2.20	0.00	0.00	0.00	1.39
Marjoram oil 0.25%	2.75	2.47	2.24	2.00	0.00	0.00	1.58
Marjoram oil 0.50 %	2.45	2.29	2.15	0.00	0.00	0.00	1.15
Rosemary oil 0.25 %	3.50	3.25	3.10	2.50	0.00	0.00	2.06
Rosemary oil 0.50 %	3.21	2.95	2.46	0.00	0.00	0.00	1.44
Sage oil 0.25 %	2.93	2.76	2.13	0.00	0.00	0.00	1.30
Sage oil 0.50 %	2.59	2.21	0.00	0.00	0.00	0.00	0.80
	3.19	2.98	2.26	1.23	0.67	0.79	1.85
L.S.D $_{0.05}$ **A**	0.08						
B	0.12						
AB	0.28						

A= Treatments (in the same column), **B** = Storage period (in the same row), **AB**= Interaction

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